Review

Potential of *Aspergillus flavus* genomics for applications in biotechnology

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Aspergillus flavus **is a common saprophyte and opportunistic pathogen that produces numerous secondary metabolites. The primary objectives of the *A. flavus* genomics program are to reduce and eliminate aflatoxin contamination in food and feed and to discover genetic factors that contribute to plant and animal pathogenicity. *A. flavus* expressed sequence tags (ESTs) and whole-genome sequencing have been completed. Annotation of the *A. flavus* genome has revealed numerous genes and gene clusters that are potentially involved in the formation of aflatoxin and other secondary metabolites, as well as in the degradation of complex carbohydrate polymers. Analysis of putative secondary metabolism pathways might facilitate the discovery of new compounds with pharmaceutical properties, as well as new enzymes for biomass degradation.**

Introduction

Aspergillus flavus is one of the most frequently isolated mold species in agriculture and medicine. It is a common soil inhabitant and a weak plant pathogen that infects a broad range of important agricultural crops, including both monocots and dicots [1,2]. As a contaminant of stored grains and other crops, *A. flavus* produces aflatoxins, the most potent naturally occurring toxic and hepatocarcinogenic compounds [3]. Consumption of aflatoxin-contaminated foods and feeds causes acute toxicity and long-term carcinogenicity [4]. *A. flavus* also can cause direct infection and systematic disease in humans. After *A. fumigatus*, *A. flavus* is the second leading cause of invasive and non-invasive aspergillosis in immunocompromised patients [5]. Because of its capacity to produce aflatoxins and to cause disease, *A. flavus* has a largely negative reputation. However, *A. flavus* might not always be as bad as we thought. It is closely related to *A. oryzae*, a non-toxigenic mold that has been used in Asian food fermentations for over a thousand years [6] (see Box 1). Furthermore, *A. flavus* produces a whole array of secondary metabolites in addition to aflatoxins, and these unknown secondary compounds can be explored for possible pharmaceutical use. Just as importantly, *A. flavus* is capable of degrading numerous complex organic polymers and is an effective recycler in the biosphere. These abilities make it a good target organism for finding novel capacities for turning waste plant polymers into biofuels. The technological breakthroughs in fungal genomics and other '-omics' technologies now allow scientists to use bioinformatics and data analysis tools for genomic data mining, transcriptomics and gene-profiling studies, which allow us to access the rich physiological repertoire of this versatile and ubiquitous species [7,8]. In addition, the availability of several other *Aspergillus* genomes, including *A. fumigatus* [9], *Neosartorya ficheri* (anamorph *A. fisheri*), *A. oryzae* [6], *A. nidulans* [10] and *A. niger* [11], makes it possible to use comparative genomics to study the basic fungal biology, mycotoxin-producing potential, genetic regulation and evolution of these *Aspergillus* species. Comparative genomics data can be leveraged to characterize species-specific biosynthetic processes. Similarly, microarrays and proteomics technology allow us to study comparative gene expression levels with controlled variation of environmental parameters. *A. flavus* genomics can also shed light on the big question of why *Aspergilli* produce mycotoxins with potential links to their fitness property. The current progress of *A. flavus* genomics and biotechnological applications for *A. flavus* in the development of pharmaceutical drugs, resistant commercial crops in agriculture and biofuel production through hydrolytic enzyme discovery are reviewed and discussed below.

Aspergillus flavus genomics for elucidation of the aflatoxin pathway and regulation

The first genomic effort completed at The Food and Feed Safety Research Unit of Southern Regional Research Center, USDA/ARS, was the *Aspergillus flavus* Expressed Sequence Tags (ESTs) project. A total of 7218 unique *A. flavus* EST sequences were identified from 19 618 ESTs generated (see the National Center for Biotechnology Information Genome Wide Association Database [http://www.ncbi.nlm.nih.gov/] and The Computational Biology

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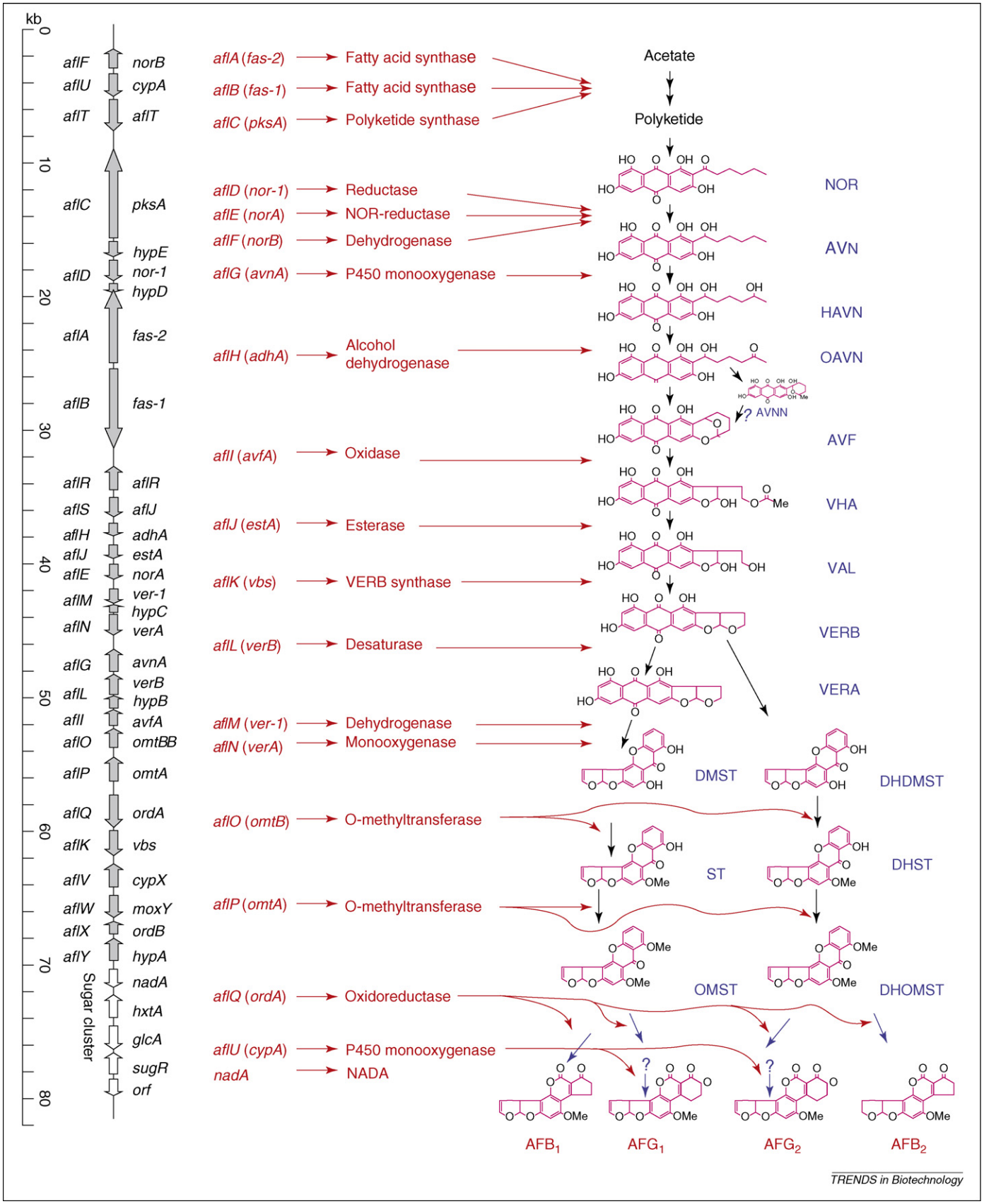

Figure 1. The aflatoxin biosynthetic pathway and clustered genes. The genes with their new and old names are shown on the left. The generally accepted pathway for aflatoxin biosynthesis is presented on the right. The vertical line represents the 82 kb aflatoxin biosynthetic pathway gene cluster and sugar utilization gene cluster in *A. parasiticus* and *A. flavus*. The new gene names are given on the left of the vertical line and the old gene names are given on the right. Arrows along the vertical line indicate the direction of gene transcription. The ruler on the far left indicates the relative sizes of these genes in kilobase pairs. Arrows in the pathway scheme indicate the connections from the genes to the enzymes they encode, from the enzymes to the bioconversion steps they are involved in and from the intermediates to the products in the aflatoxin bioconversion steps. Abbreviations: NOR, norsolorinic acid; AVN, averantin; HAVN, 5′-hydroxy-averantin; OAVN, oxoaverantin; AVNN, averufanin; AVF, averufin; VHA, versiconal hemiacetal acetate; VAL (VHOH), versiconal; VERB, versicolorin B; VERA, versicolorin A; DMST, demethylsterigmatocystin; DHDMST,

Box 1. *Aspergillus oryzae* and *Aspergillus flavus*: a Janus-faced complex of closely related molds

Aspergillus oryzae was domesticated for food production in China at least two millennia ago. It is an essential part of several complex food fermentations, of which rice wine, soy sauce and fermented soy bean paste (miso) are best known. All of the *A. oryzae* food fermentations involve a two-stage process in which the first step is an aerobic 'koji process'. Subsequent fermentations involve yeasts and lactic acid bacteria [63,64]. The koji process was the inspiration for the first U.S. patent on a microbial enzyme, awarded in 1894 to a Japanese-born chemist named Jokichi Takamine for a crude amylase preparation from *A. oryzae* grown on wheat bran [65]. More than 100 years later, *A. oryzae* remains one of the most important production organisms in commercial enzymology, especially in Japan where *A. oryzae* has been called 'the national mold'. *A. oryzae* does not produce aflatoxins and has become a domesticated species that is rarely encountered in nature. It is not associated with plant or animal disease. *A. flavus* can be viewed as the 'evil twin' of *A. oryzae*. Although morphologically almost indistinguishable, *A. flavus* is widespread in nature. It produces aflatoxins, invades crops in the field and causes direct infections in humans and animal species [5].

The profoundly different economic impact of these two molds is all the more surprising in that they are very difficult to distinguish. Ever since the first major monograph on the genus *Aspergillus* was published in 1926 by Thom and Church [60], the remarkable morphological similarity between *A. flavus* and *A. oryzae* has been noted. Taxonomic distinctions are based on minor differences in anatomical features of the spore and certain culture characteristics when grown under standardized conditions [66]. Classical mold taxonomists grouped them together in the '*Aspergillus flavus* group', whereas cotemporary scientists group them together under the rubric of the '*Aspergillus* Section Flavi'.

How can two species be so different and yet so much alike? Taxonomists, food scientists and industrial mycologists have asked this question for a long time. The *A. oryzae* genome is already available [6] and the *A. flavus* sequencing has been completed [13]. The elucidation of the DNA sequence and cognate functional genome studies for *A. flavus* should bring new answers.

and Functional Genomics Laboratory Gene Index Project [http://compbio.dfci.harvard.edu/tgi]) [12]. More recently, a whole-genome sequencing effort has been completed at The J. Craig Venter Institute (JCVI); the sequence was accomplished using a shotgun approach and the Sanger sequencing protocol and contained fivefold coverage [13,14]. Genome assembly indicates that the 16 largest scaffolds, which correspond to the 16 predicted arms of the eight *A. flavus* chromosomes, represent over 99.6% of the genome and 99.6% of the predicted homologous genes [13,14]. The estimated genome size of *A. flavus* is 36.8 Mb, which is similar to that of *A. oryzae* (36.7 Mb) [6] (see Box 1) but larger than the genomes of *A. nidulans* (30.1 Mb) and *A. fumigatus* (29.4 Mb). The sequence data have been deposited to the GenBank Database (http://www.ncbi.nlm.nih.gov/, with the genome accession number: AAIH00000000, scaffolds EQ963472-EQ966232) and are also available through the *Aspergillus flavus* website (http://www.aspergillusflavus.org).

A preliminary survey of the *A. flavus* genome indicates that there are over 12 000 functional genes. Bioinformatic analysis and EST data reveal numerous categories of genes encoding for enzymes that are possibly involved, either directly or indirectly, in the production of aflatoxin or other secondary metabolites, such as in signal transduction, pathogenicity, virulence and fungal development [12]. Previous molecular cloning of the aflatoxin biosynthetic cluster in *A. flavus* and *A. parasiticus* led to the identification of 29 genes within an 82 kb DNA region [15]. By mapping of the ESTs to this cluster region of the genome, an additional five new transcripts (*hypA*, *hypB*, *hypC*, *hypD* and *hypE*) were identified that might have involvement in aflatoxin biosynthesis (see Figure 1). Acquisition of these gene sequences facilitated the manufacture of microarrays for functional genomics studies. Four formats of *A. flavus* microarrays and two formats of *A. oryzae* microarrays have been designed and constructed (Table 1) [16–25]. These microarray resources provide a platform for functional genomic studies, and hundreds of fungal genes have been identified that are significantly up- or downregulated under various growth conditions [16–19,21,23,26–29].

The aflatoxin biosynthetic pathway has been studied for over 40 years and is one of the best understood fungal secondary metabolic pathways (see Figure 1). Up to now, at least 34 genes have been identified as members of the aflatoxin pathway gene cluster [15]. Most of their functions in the pathway have been elucidated. The aflatoxin regulatory gene (*aflR*) encodes a positive regulator, AFLR, which activates aflatoxin pathway gene transcription [30], whereas the *aflS* (old name *aflJ*) gene encodes a protein factor, AFLS, which was found to be involved in the regulation of transcription [31,32]. Temperature affects aflatoxin production in *A. flavus* by altering the transcriptional profile [29]. An aflatoxin-producing strain subjected to a temperature shift from 28 °C to 37 °C quickly stops aflatoxin biosynthesis. Transcript profiling at 28 °C versus 37 °C identified a total of 144 genes differentially expressed, of which 103 were more highly expressed at 28 °C. Most of the aflatoxin pathway genes were more highly expressed at 28 °C relative to 37 °C. However the regulatory genes *aflR* and *aflS* were relatively constant at both temperature conditions, suggesting that the failure to produce aflatoxin at 37 °C is not due to the effect of temperature on the transcription of the pathway regulatory genes, *aflR* and *aflS*, but due possibly to non-functionality at higher temperatures or alteration in the interaction between AFLS and AFLR [32].

The regulatory genes that directly control aflatoxin biosynthesis, such as *aflR* [30] and *aflS* (*aflJ*) [31,32] are well studied. The genes *laeA* (*loss of aflR expression A*) [33] and *veA* (one of the velvet complex genes, connecting light-responding developmental regulation and control of secondary metabolism) [34] regulate fungal development and affect both aflatoxin and sterigmatocystin production [21]. Unlike *aflR* and *aflS*, the *laeA* and *veA* genes are located outside the known aflatoxin pathway gene cluster. In *A. flavus* (NRRL 3357), *A. nidulans*, *A. oryzae* (RIB40) and *A. fumigatus* (CE10), the *laeA* gene is well conserved. In *A. nidulans*, light stimulates asexual development and simultaneously represses sexual development and sterigmatocystin production. Bayram *et al.* [35] have identified a heterotrimeric VelB–VeA–LaeA complex in *A. nidulans*

dihydrodemethylsterigmatocystin; ST, sterigmatocystin; DHST, dihydrosterigmatocystin; OMST, *O*-methylsterigmatocystin; DHOMST, dihydro-*O*-methylsterigmatocystin; AFB_1, aflatoxin B_1; AFB_2, aflatoxin B_2; AFG_1, aflatoxin G_1; and AFG_2, aflatoxin G_2.

Table 1. Whole-genome arrays available for *A. flavus* and *A. oryzae*

Organism	Strain	Type of array	No. of genes	Refs
A. flavus	NRRL 3357	Spot gene array	753	[16]
A. flavus	NRRL 3357	gDNA amplicon array	5031	[17–19, 21,23]
A. flavus	NRRL 3357	Affymetrix oligo array	~12 000	[20]
A. flavus	NRRL 3357	cDNA oligo array	~12 000	[22]
A. oryzae	RIB40	cDNA amplicon array	2070	[24]
A. oryzae	RIB40	cDNA oligo array	~12 000	[25]

that acts as a developmental switch in controlling both morphology and secondary metabolite formation. Gene-profiling studies on the *veA* gene in a *veA*-disrupted mutant using the 5031 gene amplicon array (Table 1) in *A. flavus* revealed 684 genes whose expression changed significantly over time. A group of 115 genes showed greater expression in the wild-type than in the *veA* mutant strain [21]. Gene-profiling studies on the *laeA* gene using an *laeA*-disrupted mutant in *A. fumigates* showed that *laeA* influences the expression of at least 9.5% of the genome (943 of 9626 genes in *A. fumigatus*) but positively controls expression of 20% to 40% of major classes of secondary metabolite biosynthesis genes [36]. Studies at the genome scale, using *A. flavus laeA*-knockout mutants, are well underway. These studies will help in identifying gene networks regulated by these regulatory genes. The elucidation of their regulatory mechanisms could be explored for intervention of aflatoxin production and fungal invasion in agricultural crops.

A. flavus genomics for identification of novel secondary metabolites

In addition to aflatoxins, *Aspergillus* species could also produce beneficial secondary metabolites, such as antibiotics and other pharmaceuticals [37]. For example, *A. terreus* is known to produce lovastatin, a potent cholesterol-lowering drug [38] that is one of the most profitable products in medicine, generating revenues in excess of US$1 billion annually. Other *Aspergillus* and *Penicillium* species secrete antibiotics (penicillin, cephalosporin and gliotoxin), antifungals (griseofulvin) and antitumor agents (terrequinone A) [39,40]. *A. flavus* is known to be a prolific producer of secondary metabolites, including the mycotoxins aflatoxin, sterigmatocystin, cyclopiazonic acid, 3-nitropropionic acid and aspertoxin [41]. It might also be a potential source of new therapeutics.

The discovery of novel secondary metabolite biosynthesis pathways is now facilitated by bioinformatics tools such as the web-based software called SMURF (secondary metabolite unique regions finder, available at http://www.jcvi.org/smurf). Using these tools, both the *A. flavus* and *A. oryzae* genomes [6,14] were shown to contain several hitherto undescribed secondary metabolite pathways. Experimental work to express these clusters and to screen new metabolites for their pharmaceutical effects is a promising avenue for drug research.

Identification of the 'backbone' genes involved in secondary metabolite biosynthesis

Annotation of the *A. flavus* genome indicates that it has more secondary metabolite biosynthetic pathways than other sequenced *Aspergillus* species [42]. SMURF-based analysis showed that its genome contains at least 70 'backbone' genes, which often encode enzymes catalyzing the first step in many secondary metabolite biosynthesis pathways. The number and categories of the backbone genes are listed in Table 2.

Table 2. Enzymes that are putatively involved in secondary metabolite biosynthesis and transport in *A. flavus* and *A. oryzae* [14,61,62]

Enzymes	Abbreviation	*A. flavus*	*A. oryzae*
Nonribosomal peptide synthase	NRPS	18	16
Nonribosomal peptide synthase-like	NRPS-like	14	16
Polyketide synthase	PKS	25	27
Polyketide synthase-like	PKS-like	3	0
Hybrid PKS–NRPS enzyme	HYBRID	2	2
Prenyltransferases	PTR	8	8
ABC transporters	ABC	76	79
MFS transporters	MFS	459	468
Cytochrome P450 enzymes	P450	126	137

Analysis of the domain content of the backbone genes in the *A. flavus* genome might provide important clues to the types of molecules produced by the fungus, such as polyketides synthases (PKSes), non-ribosomal peptide synthases (NRPSes) and terpenes, among others, as well as their possible chemical structures. Additional information can be extracted from any adjacent genes, which together with backbone genes form the secondary metabolism gene clusters. The adjacent genes often encode enzymes, such as hydroxylases, oxidases and methylases, that catalyze later steps in secondary metabolite biosynthesis. Transporters and transcriptional regulators are also often encoded within these gene clusters. A preliminary analysis of these backbone genes and their corresponding clusters is given below.

Polyketide synthases (PKSes)

Of the 24 putative *A. flavus* PKSes, only *aflC (pksA)* has been experimentally characterized. Not much is known about the remaining 23 putative *A. flavus* PKS enzymes. Among them, 19 can be classified as Type I enzymes, that is, they are multidomain, single module enzymes with significant sequence similarity to fatty acid synthases [43]. On the basis of the domains present and their phylogeny, these PKS enzymes can be categorized into sequential, modular and iterative enzymes, which carry out and control chain initiation, elongation, folding and cyclization [44]. Apart from Type I PKSes, the *A. flavus* genome has four Type III PKS genes that resemble the chalcone synthase genes found in plants and are orthologous to four genes identified in *A. oryzae* [45]. Type III PKS genes seem to be absent from other sequenced *Aspergillus* species, although they have been detected in some other filamentous fungi and might be involved in biosynthesis of so far undiscovered compounds.

Nonribosomal peptide synthases (NRPSes)

None of the 18 putative *A. flavus* NRPS encoding genes has been experimentally characterized so far. Three NRPSes seem to be orthologous to well-characterized genes, such as *sidD* (*sid2*), which is involved in siderophore biosynthesis

in *A. fumigatus* [46], *pes1* [47], which mediates resistance to oxidative stress in *A. fumigatus*, and *pcbAB*, which is involved in penicillin biosynthesis in *Penicillium chrysogenum* [48]. Another putative *A. flavus* NRPS gene is distantly related to *A. fumigatus gliP*, which is a bimodular NRPS involved in biosynthesis of gliotoxin, a well-studied virulence factor involved in immunosuppression and activity against lymphocytes and macrophages. The remaining *A. flavus* NRPSes might be responsible for biosynthesis of hitherto uncharacterized peptide compounds.

Hybrid PKS–NRPS

The *A. flavus* genome also contains two hybrid genes that encode a PKS module followed by a single NRPS module. Hybrid PKS–NRPS enzymes found in other fungal species are involved in biosynthesis of mycotoxins such as fusarin C and beneficial compounds such as equisetin [49], the HIV-1 integrase inhibitory tetramic acid. Both *A. flavus* hybrid genes share regions of similarity with equisetin synthetase *eqiS*, which is involved in biosynthesis of equisitin in *Fusarium heterosporum* [49,50]. Furthermore, it has been suggested that one of these hybrid PKS–NRPS enzymes might be responsible for the production of cyclopiazonic acid, an indole tetramic acid derived from tryptophan, dimethylallyl diphosphate and malonate secreted by *A. flavus* and some other *Aspergillus* species.

A. flavus genomics for the discovery of cellulosic degrading enzymes

The non-fastidious and sometimes aggressive growth properties of *A. flavus* make this fungus a suitable candidate for exploration in the search for new microbes for biofuel production. The first objective of The Genomics: Gas to Liquid (GTL) project (U.S. Department of Energy) is the discovery of new cellulosic enzymes via a genomics strategy (http://genomicsgtl.energy.gov/research/DOEUSDA/index.shtml) [51]. Preliminary annotation of the *A. flavus* genome indicates that there are several categories of genes encoding pecto-cellulosic enzymes (Yu, J. *et al.*, unpublished observations). These include at least 74 hydrolases and encompass 3 cellulases, at least 6 xylanases, 12 chitinases, 9 α amylases, at least 18 pectinases, and 8 proteinases. A comparison with closely related *Aspergillus* species for which genome information is available shows similar numbers for each enzyme class. The closely related species *A. oryzae* is a food-grade organism with GRAS status (Generally Regarded As Safe by the U.S. Food and Drug Administration [FDA]) that is already widely used in industrial fermentation. Not only are the numbers of deduced hydrolytic genes the same in both species, but the sequence identities are also are up to 100% in the coding regions of each corresponding gene. Unlike *A. flavus*, *A. oryzae* does not have the ability to survive in the wild. The greater competitive ability of *A. flavus* might render it a better candidate to become an elite biofuel production strain.

Using microarrays, experiments are underway to compare gene expression in *A. flavus* strain NRRL 3357 and *A. oryzae* strain RIB 40 in solid state fermentations on wheat bran, a cellulose rich substrate. Preliminary data indicate that *A. flavus* genes encoding for cellulosic enzymes were up to two orders of magnitude more highly expressed than those in *A. oryzae* (Yu, J. *et al.*, unpublished observations). These highly expressed genes included sequences encoding amylases, glucanases, xylanases, chitinases, glycosyl hydrolases, sugar transporters and major facilitator superfamily (MSF) proteins. Though the same genes exist in both species, *A. flavus* is able to express those genes at a higher level under the same conditions in response to the fiber-rich solid medium, indicating different regulatory mechanisms. If targeted regulatory components can be identified through gene profiling, a highly efficient biodegrader could be engineered for waste treatment and/or bioenergy production. Current technologies allow the delivery of more than one gene by fungal transformation [52] and have the potential to yield a highly efficient filamentous degrader for biofuel production.

A. flavus genomics for understanding the mechanisms of fungal infection

In addition to the burden of disease, *A. flavus* causes aflatoxin contamination of foods and its abundant airborne spores cause allergies. *A. flavus* is the second leading cause of invasive aspergillosis in immunocompromised human patients in Europe and the USA. Many localized cases of aspergillosis, such as sinus and cuteaneous infections, are also caused by *A. flavus* [53,54]. To date, most studies aiming to determine the infection mechanism of *Aspergillus* species have focused on *A. fumigatus*. These findings are likely to be informative for *A. flavus*. In *A. fumigatus* neither unique gene products nor exclusive biochemical pathways have been identified to account for disease-producing ability. The search for so called 'virulence determinants' has proven elusive, and there is now the general consensus that the potential for pathogenicity is highly multifactorial [55].

A. flavus is usually categorized as either a saprophyte or an opportunistic human pathogen. In reality, the fungus lives more than a dual life. *A. flavus* has the extraordinary capacity to cause disease in diverse plants, in insects and in numerous vertebrate species. In other words, *A. flavus* is a pan-kingdom pathogen. In plants, *A. flavus* infection is usually correlated with drought, insect damage and temperature stress. Once established in a plant, *A. flavus* is able to colonize seeds and to compete strongly with other endophytes and mycoflora.

Infection of maize seeds might occur directly or through wounds created by insects [56,57]. In both cases, the developing embryo and its surrounding tissue are colonized first [58]. Only after these tissues are decayed does the fungus begin to degrade the starchy endosperm, a somewhat surprising chronology. One would expect the endosperm, which undergoes programmed cell death early in the development of maize seeds [59], to be the most susceptible to degradation by a saprophytic fungus.

Whole-genome sequences and DNA microarrays of *A. flavus* and maize are available for genome analysis and gene-profiling studies. The knowledge obtained through such microarray experiments can help plant breeders to develop commercial maize varieties with resistance to *A. flavus* infection by genetic engineering or conventional breeding strategies.

Conclusions

One of the most salient findings from the *A. flavus* genome project is its close sequence similarity and genomic architecture to that of the *A. oryzae* genome [6]. Since visible phenotype is a manifestation of many genes and pathways acting together, the high genomic identity merely confirms what taxonomists have known since their earliest descriptions of the *A. flavus-oryzae* group of yellow-green aspergilla [60]. The morphological, physiological and genomic correspondence between the species is all the more remarkable because of their differing economic repercussions in human society. To reiterate, *A. flavus* not only produces aflatoxin, a highly carcinogenic mycotoxin, but is also a pan-kingdom pathogen capable of causing serious diseases in plants, insects and vertebrates. By contrast, *A. oryzae* does not produce aflatoxins, nor does it infect plants or animals. *A. oryzae* is essential in several major Asian food fermentations and is 'generally regarded as safe' by the FDA. How can two species be so alike yet so different?

With increasingly sophisticated '-omics' approaches, our understanding of this economically important group of molds has been expanded significantly, but we are still not able to answer this question; without a doubt, the high degree of DNA homology and synteny between the two species indicates an extremely close evolutionary relationship.

In fact, many important questions remain unanswered. Although large numbers of deduced genes in both species still cannot be assigned to functional classes, most genes found in both species are identical. The acquisition of genome-wide data for *A. flavus* and *A. oryzae* has not enlightened us about the mechanics of pathogenicity and competitiveness, the specific selective influences associated with the domestication of *A. oryzae* nor the successful ecosystem dynamics exhibited by *A. flavus* [13]. In a broader context, genomics is a tool too reductionist to illuminate how some lineages are adapted to agriculture while others are highly domesticated. We still do not understand why *A. oryzae* is only rarely found in nature or as a cause of disease but *A. flavus* is prevalent as a contaminant in agriculture and in modern hospitals.

These genome data do permit us to make strong inferences about the comparative biology of these molds and to reconstruct possible scenarios for the evolution of secondary metabolite clusters, the enzymes involved in biomass degradation and in other important pathways, and open new avenues for research. The future promises even more of a data deluge as transciptome, proteome and metabolome profiling become increasingly available. Biologists trained in traditional microbiology, biochemistry and plant pathology will have to become adept at systems biology to take advantage of these tools. But we must not forget that these *in silico* approaches are no substitute for the experimental studies necessary for us to understand *A. flavus*, *A. oryzae* and other *Aspergillus* species in their ecological contexts. When interdisciplinary teams combine wet laboratory, field and '-omics' approaches to studying *A. flavus*, it is highly likely that the next decades will see an explosion of informative and exploitable research findings for this economically destructive yet potentially useful mold.

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